Original: Japanese
Provisional translation

Analytical Method for Fosfomycin (Animal and Fishery Products)

1. Analyte

Fosfomycin

2. Application

Animal and fishery products

3. Instrument

Liquid chromatograph-tandem mass spectrometer (LC-MS/MS)

4. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Ammonia solution: 25% ammonia solution (special grade)

10 mmol/L ammonium acetate solution (pH 3): Weigh 0.77 g of ammonium acetate, dissolve it in approximately 900 mL of water, adjust the pH to 3 with acetic acid and add water to make a 1 L solution.

Tertiary amine-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (150 mg): A polyethylene tube of 8–9 mm in inside diameter packed with 150 mg of weakly basic anion-exchange resin or other cartridges with equal separation characteristics.

Reference standard of fosfomycin disodium salt: Contains not less than 95% of fosfomycin disodium salt.

5. Procedure

1) Extraction

Add 100 mL of methanol to 10.0 g of sample, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 10 mL aliquot of the solution, concentrate at below 40°C and remove the solvent. Dissolve the residue in 10 mL of water, add 20 mL of *n*-hexane, shake and wash. Take the aqueous layer, add 10 mL of water to the *n*-hexane layer, take the aqueous layer and combine it with the previous aqueous layer.

2) Clean-up

Inject 10 mL each of methanol and water into an octadecylsilanized silica gel cartridge (2 g) sequentially and discard each effluent. Inject 10 mL each of methanol, 2 vol% formic acid, and water into a weakly basic anion-exchange resin cartridge (150 mg) sequentially and discard each effluent. Connect the weakly basic anion-exchange resin cartridge to the bottom of the octadecylsilanized silica gel cartridge, transfer the solution obtained in 1) and discard the effluent. Then, add 10 mL of water and discard the effluent. Detach the

octadecylsilanized silica gel cartridge, inject 10 mL each of methanol and water into a tertiary alkylamine-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge sequentially and discard each effluent. Then, add 20 mL of 10 mmol/L ammonium acetate solution (pH 3), concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in a mixed solution of ammonia solution, 20 mmol/L ammonium hydrogen carbonate solution and acetonitrile (1:250:250) to make exactly 5 mL, and use this solution as the test solution.

6. Calibration curve

Dissolve the reference standard of fosfomycin disodium salt in water to make 1,000 mg/L stock standard solution of fosfomycin. Dilute the stock standard solution with a mixed solution of ammonia solution, 20 mmol/L ammonium hydrogen carbonate solution and acetonitrile (1:250:250) appropriately and prepare standard solutions of several concentrations. Inject each solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the concentration of fosfomycin in the test solution corresponding to 0.05 mg/kg in the sample is 0.005 mg/L.

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of fosfomycin from the calibration curve prepared in **6**.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example 1)

Column: Silica gel bonded with carbamoyl group, 2.1 mm inside diameter, 150 mm in length and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: A mixed solution of ammonia solution and 10 mmol/L ammonium hydrogen carbonate solution (1:500)

Mobile phase B: A mixed solution of ammonia solution, 50 mmol/L ammonium hydrogen carbonate solution, and acetonitrile (1:100:400)

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	0	100
10	100	0
16	100	0

Ionization mode: ESI (−)

Major monitoring ions (*m/z*): Precursor ion 137, product ions 79, 63

Injection volume: 2 μL

Expected retention time: 5 min

10. Limit of quantification

0.05 mg/kg

11. Explanatory note

1) Outline of analytical method

The method consists of extracting fosfomycin from sample with methanol, washing with *n*-hexane, cleaning up with an octadecylsilanized silica gel cartridge and a tertiary alkylamine-modified divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge and quantifying and confirming using LC-MS/MS.

2) Notes

i) When the analytical method for fosfomycin using LC-MS/MS was developed, the following monitoring ions were used:

for quantitative ions (*m/z*): precursor ion 137, product ion 63

for qualitative ions (*m/z*): precursor ion 137, product ion 79

ii) A chromatogram of qualitative ions can be affected by interference peaks in some foods. Therefore, if qualitative confirmation with qualitative ions is difficult, confirm using the following measurement conditions (Example 2). In addition, when the measurement is performed under the measurement condition (Example 2), the standard and test solutions for measurement are prepared using a mixed solution of ammonia water and 10 mmol/L ammonium hydrogen carbonate solution (1:500).

Measurement conditions (Example 2)

Column: porous graphitic carbon, 2.1 mm inside diameter, 150 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: A mixed solution of ammonia solution and 10 mmol/L ammonium hydrogen carbonate solution (1:500)

Ionization mode: ESI (−)

for quantitative ions (*m/z*): precursor ion 137, product ion 63

for qualitative ions (*m/z*): precursor ion 137, product ion 79

Injection volume: 5 μL

Expected retention time: 4 min

iii) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, cattle milk and yellowtail.

12. Reference

None

13. Type

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